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MINIREVIEW

Response to Iron Deprivation in *Saccharomyces cerevisiae*[▽]

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Iron is an essential nutrient for almost every organism because iron cofactors, such as heme and iron-sulfur clusters (ISC), are required for the activity of numerous cellular enzymes involved in a wide range of cellular processes. Although iron is a very abundant metal in the earth's crust, it has a low bioavailability. In aerobic environments, iron is largely present in the oxidized, ferric form, which is poorly soluble at neutral pH. Thus, iron can be limiting for growth, and single-celled organisms and plants have evolved sophisticated strategies for acquiring iron from the environment. In humans, iron is absorbed poorly from the diet, especially if the diet is entirely plant based and low in ascorbic acid. Iron deficiency is the most common nutritional deficiency in the world and is a significant public health problem, especially among children and women of childbearing age (68). The most obvious manifestation of iron deficiency in humans is anemia, but iron deficiency has adverse effects on the immune system and cognitive development as well. Although the pathogenesis of anemia in iron deficiency is well understood, other manifestations of iron deficiency are not understood at the cellular or metabolic level. Conversely, the accumulation of excess iron within cells is a feature of several human diseases, both acquired and inherited, and the mechanisms by which excess iron damages cells are far from clear (3, 17, 35, 40). Work with the simple eukaryote *Saccharomyces cerevisiae* has begun to reveal how cells adapt to changes in the availability of iron.

The budding yeast *S. cerevisiae* can thrive in environments in which the bioavailable iron is extremely scarce or overly abundant, and it can survive tremendous fluctuations in iron availability. This review focuses on the strategies exhibited by this organism as it responds to the depletion of iron in its environment. This response consists of the following three aspects: (i) activation of systems of iron uptake, (ii) mobilization of intracellular stores of iron, and (iii) metabolic adaptations to iron limitation. Furthermore, the response appears to be graded, that is, lesser degrees of iron deprivation trigger a modest iron deficiency response, while greater degrees trigger a greater response. Much of this response is mediated through changes in transcription, but additional, posttranscriptional mechanisms are also employed.

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AFT1P AND AFT2P ARE THE PRINCIPAL MEDIATORS OF THE TRANSCRIPTIONAL RESPONSE TO IRON DEPLETION

The primary response to iron depletion in yeast is the transcriptional activation of a set of genes under the control of the iron-dependent transcription factor Aft1p (Fig. 1) (64, 65). Aft1p is constitutively expressed, and when intracellular iron is abundant, Aft1p is localized to the cytosol and does not activate transcription (66). When iron levels are low, Aft1p accumulates in the nucleus, where it binds to DNA and activates transcription. Aft1p appears to sense intracellular iron levels, but whether Aft1p directly binds iron is not known. Aft1p is thought to continuously cycle in and out of the nucleus. Transport of Aft1p into the nucleus is dependent on the karyopherin Pse1p and occurs through the interaction of Pse1p with two nonclassical nuclear localization sequences within Aft1p (59). The binding of Pse1p to these sequences is not regulated by iron, and the effects of iron on Aft1p localization do not appear to be exerted through nuclear import.

Elevated intracellular iron levels trigger the nuclear export of Aft1p, and numerous cellular proteins are required for this export. A series of conserved cysteine desulfurases, scaffold proteins, iron chaperones, and thioredoxins located in mitochondria are required for the assembly of ISC prior to their insertion into enzymes that require these cofactors. The mitochondrial ISC assembly machinery is required for the iron-dependent nuclear export of Aft1p. Deletion of the mitochondrial monothiol glutaredoxin Grx5p (1), depletion of mitochondrial frataxin (Yfh1p), or depletion of glutathione leads to a loss of both mitochondrial ISC assembly and iron-dependent inactivation of Aft1p (4, 52). The mitochondrial inner membrane transporter Atm1p transports a compound that is required for both cytosolic ISC maturation and Aft1p inactivation, yet the cytosolic ISC machinery itself is not required for Aft1p inactivation. Depletion of the cytosolic ISC proteins Nar1p, Cfd1p, and Nbp35p does not induce the expression of Aft1-regulated genes and hence does not prevent the iron-induced inactivation of Aft1p.

The nuclear monothiol glutaredoxins Grx3p and Grx4p are required for the inactivation and nuclear export of Aft1p, and deletion of both Grx3p and -4p results in constitutive expression of Aft1p target genes (37, 46). Both glutaredoxins can bind to Aft1p, and a conserved cysteine residue in the glutaredoxin active site is required for binding and inactivation. Grx3p and -4p binding does not appear to be regulated by iron, however, and cannot explain the iron-dependent inactivation of Aft1p. Ueta and colleagues recently reported that the amino-

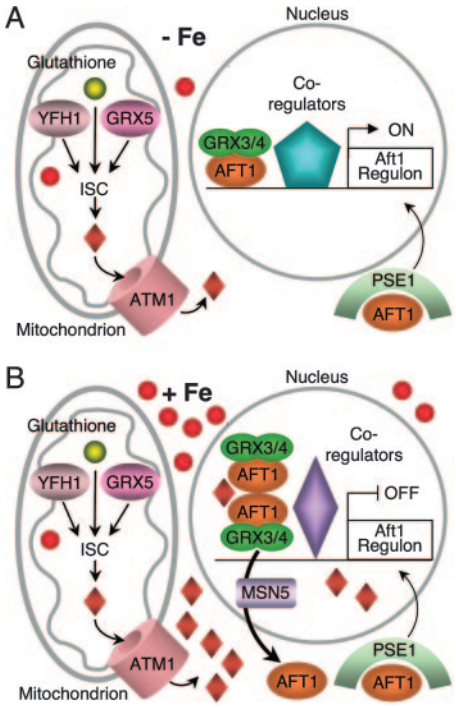

FIG. 1. Iron-dependent transcriptional regulation in *Saccharomyces cerevisiae*. (A) Activation of Aft1p under conditions of iron deprivation. The nuclear importin Pse1p mediates Aft1p translocation into the nucleus. Aft1p forms a complex with Grx3p and Grx4p, binds to DNA, and activates transcription. Although complex formation is not regulated by iron, it is not known whether complex formation occurs exclusively in the nucleus or also in the cytosol. "Coregulators" represent the numerous coactivators and corepressors that contribute to the regulation of the Aft1p regulon. These include the mediator complex, Snf1p/Snf4p, Ssn6, Nhp6p, Tup1, Hda1p, Cti6p, and heme. (B) Regulation of Aft1 activity under iron-replete conditions. Yfh1p, Grx5p, and glutathione are required for the production of ISC and the formation of an unknown compound that is a substrate for Atm1p. This compound is exported from mitochondria and may possibly be targeted to the nucleus. Under iron-replete conditions, Aft1p forms dimers that are recognized by the nuclear exportin Msn5p and lead to the accumulation of Aft1p in the cytosol. In a hypothetical model for the regulation of Aft1p, the production of the substrate for Atm1p is proportional to cellular iron levels. This substrate accumulates in the nucleus and leads to the dimerization of Aft1p, perhaps through the formation of a mixed disulfide bridge, and the complex is exported from the nucleus.

and carboxyl-terminal domains of Aft1p exhibit an intermolecular interaction in the presence of iron and that iron induces the formation of dimers of Aft1p (58). These interactions require cysteine residue 291 in both binding partners, and this residue is mutated in a constitutively active allele of *AFT1*. This iron-dependent interaction allows the nuclear exportin Msn5p to bind to Aft1p and mediate its transfer to the cytosol. These observations suggest a model in which, in the presence of iron, dimers of Aft1p form a mixed disulfide bridge, perhaps involving Grx3p and -4p and some product of the ISC machinery. Increasing levels of cellular iron could be reflected in an increase in the amount of the compound that is produced by the ISC machinery and transported by Atm1p (Fig. 1). However, no direct experimental evidence is currently available to support or refute this model.

Heme also plays a role in the transcriptional activation of Aft1p target genes, as disruption of heme biosynthesis impairs the transcription of a subset of these genes in response to iron depletion (10). In the absence of heme, genes encoding the high-affinity ferrous iron transport complex are repressed, and this repression requires Tup1p and Hda1p (9). Other Aft1p targets, such as the genes encoding the siderophore transporter Arn1p and the cell wall protein Fit1p, are not repressed in the absence of heme and require Cti6p to escape repression (9, 45). Because both heme synthesis and ferrous iron uptake (but not siderophore uptake) are oxygen-dependent processes, this requirement for heme may allow the cell to coordinate iron uptake with oxygen availability. The activation of some Aft1p target genes also depends on Tup1p (9, 28), and the global regulators Ssn6p and Nhp6p interact with Aft1p to enhance transcription at the *FRE2* promoter (13). The mediator complex is an evolutionarily conserved coregulator of RNA polymerase II transcription, and the modular components of the complex can have antagonistic effects on the transcription of specific genes. Cdk8p-mediated phosphorylation of a single site in the tail of the mediator complex can also specifically repress Aft1p target genes (62). Environmental factors other than iron depletion, such as the glucose depletion that occurs during the diauxic shift, also appear to activate Aft1p (16). Glucose depletion triggers a transition from fermentative to respiratory metabolism and is accompanied by increased expression of several Aft1p target genes. Both Aft1p and the Snf1p/Snf4p kinase are required for this induction. Exposure to toxic levels of cobalt also activates Aft1p, although the mechanism of Aft1p activation under such conditions is not known (56).

Nuclear Aft1p recognizes and binds to consensus sequences (PyPuCACCC) in the upstream regions of target genes (65). A paralogue of Aft1p, termed Aft2p, is 39% identical to Aft1p, recognizes similar consensus sequences, and can activate transcription of a partially overlapping set of target genes (2, 8, 50, 51). The role of Aft2p in the response to iron depletion is much less clear, however, as the transcriptional effects of Aft2p are largely inapparent unless strains are deleted for Aft1p. Similar to Aft1p, Aft2p is activated by iron depletion and directs the transcription of many Aft1p target genes as well as two genes, *SMF3* and *MRS4*, that are not targets of Aft1p. Aft2p appears to recognize a slightly different target sequence from that recognized by Aft1p. While Aft1p exhibits its strongest activation when the target is TGCACCC, Aft2p can recognize the sequences GGCACCC (present in *MRS4*) and CGCACCC (present in *SMF3*) (8, 51). The roles of these genes in vacuolar and mitochondrial iron transport, respectively, have led some investigators to suggest that Aft2p preferentially influences intracellular iron utilization (8).

THE AFT1P REGULON: GENES OF IRON UPTAKE

Aft1p activates the transcription of a specific set of genes involved in the acquisition of iron from the environment, the mobilization of stored iron, and the metabolic alterations that occur during growth under iron-limited conditions. The Aft1p and Aft2p target genes, their subcellular locations, and their functions are presented in Table 1 and Fig. 2 (8, 44, 51, 53). Seventeen genes in the Aft1p regulon are involved, either

TABLE 1. Aft1p/Aft2p target genes and the subcellular locations and functions of their products

Functional category and open reading frame	Gene name	Location	Function
Uptake of iron at the cell surface			
YDR534C	*FIT1*	Cell wall	Siderophore binding/uptake
YOR382W	*FIT2*	Cell wall	Siderophore binding/uptake
YOR383C	*FIT3*	Cell wall	Siderophore binding/uptake
YLR214W	*FRE1*	Plasma membrane	Metalloreductase
YKL220C	*FRE2*	Plasma membrane	Metalloreductase
YOR381W	*FRE3*	Plasma membrane	Siderophore reductase
YNR060W	*FRE4*	Unknown	Unknown reductase
YOR384W	*FRE5*	Unknown	Unknown reductase
YMR058W	*FET3*	Plasma membrane	Multicopper oxidase, Fe(II) uptake
YER145C	*FTR1*	Plasma membrane	Permease, Fe(II) uptake
YNL259C	*ATX1*	Cytosol	Cu chaperone, delivers Cu to Ccc2p
YDR270W	*CCC2*	Post-Golgi vesicle	Cu transport into vesicles
YHL040C	*ARN1*	Endosome, plasma membrane	Ferrichrome transport
YHL047C	*ARN2/TAF1*	Unknown	TAFC transport[b]
YEL065W	*ARN3/SIT1*	Endosome, plasma membrane	Hydroxamate siderophore transport
YOL158C	*ARN4/ENB1*	Plasma membrane	Enterobactin transport
Efflux of iron from vacuole to cytosol			
YLL051C	*FRE6*	Vacuole	Metalloreductase
YLR034C	*SMF3*[a]	Vacuole	Fe(II) transport
YFL041W	*FET5*	Vacuole	Multicopper oxidase, Fe(II) transport
YBR207W	*FTH1*	Vacuole	Permease, Fe(II) transport
Other transporters			
YGR065C	*VHT1*	Plasma membrane	Biotin transporter
YOR316C	*COT1*	Vacuole	Zn and Co storage/detoxification
YKR052C	*MRS4*[a]	Mitochondria	Mitochondrial iron import
Metabolic adaptation to low iron			
YLR205C	*HMX1*	Endoplasmic reticulum	Heme oxygenase
YLR136C	*CTH2/TIS11*	Cytosol	mRNA degradation

[a] Predominantly regulated by Aft2p. All others are predominantly regulated by Aft1p.
[b] TAFC, triacetylfusarinine C.

directly or indirectly, in the uptake of iron at the plasma membrane, and these genes allow *S. cerevisiae* to take up iron in the variety of forms that can be present in the extracellular milieu. *S. cerevisiae* takes up iron in the form of ferric and ferrous salts; low-affinity iron chelates, such as ferric citrate; and high-affinity iron chelates, such as ferric siderophores. Siderophores are a heterogeneous class of low-molecular-weight organic compounds that bind ferric iron with exceptionally high affinities and specificities (36). They are synthesized and secreted in the iron-free form by most species of bacteria and fungi. Extracellular siderophores can bind and thereby solubilize ferric iron, and the iron-siderophore complex can then be captured by cellular transport systems. Although *S. cerevisiae* does not synthesize siderophores, it can take up iron bound to a variety of these compounds.

Before iron can be taken up by the yeast cell, it must first traverse the cell wall. The Aft1p regulon includes a family of three cell wall mannoproteins that are transcribed at very high levels during iron depletion, and these are termed Fit1p, Fit2p, and Fit3p (42). These proteins contribute to the retention of the siderophore ferrichrome in the cell wall and enhance the uptake of iron bound to ferrichrome and ferrioxamine B. The mechanism by which the *FIT* genes enhance uptake is not known, but it may involve facilitating the passage of bulky iron-siderophore chelates through the cell wall or increasing the concentration of siderophores in the periplasmic space.

UPTAKE OF IRON AT THE CELL SURFACE BY THE REDUCTIVE SYSTEM

S. cerevisiae expresses two genetically separate systems for iron uptake, namely, a reductive system and a nonreductive system. Ferric salts and ferric chelates are substrates for the reductive system, while the nonreductive system exclusively recognizes siderophore-iron chelates.

Reductive uptake is a two-step process in which ferric iron is first reduced to the ferrous state and then the ferrous iron is transported into the cytosol via a high-affinity, ferrous-specific transport complex. This system of uptake has been reviewed in detail (24, 39, 63). Briefly, the reduction step is catalyzed by members of the *FRE* family of metalloreductase genes. *FRE1* and *FRE2* encode flavocytochromes that comprise the majority of surface reductase activity. They are required for growth on media that contain low concentrations of ferric iron salts, and they can catalyze the reductive release of iron from a variety of siderophores. Because reduced iron has a low affinity for siderophore ligands, reduction of ferric-siderophore complexes results in the release of ferrous iron, which can be taken up by the ferrous-specific transporter. *FRE3* encodes a plasma membrane reductase that can catalyze the reductive uptake of iron bound to hydroxamate siderophores, and Fre4p can catalyze uptake from dihydroxamate rhodotorulic acid. Each of these reductases is in-

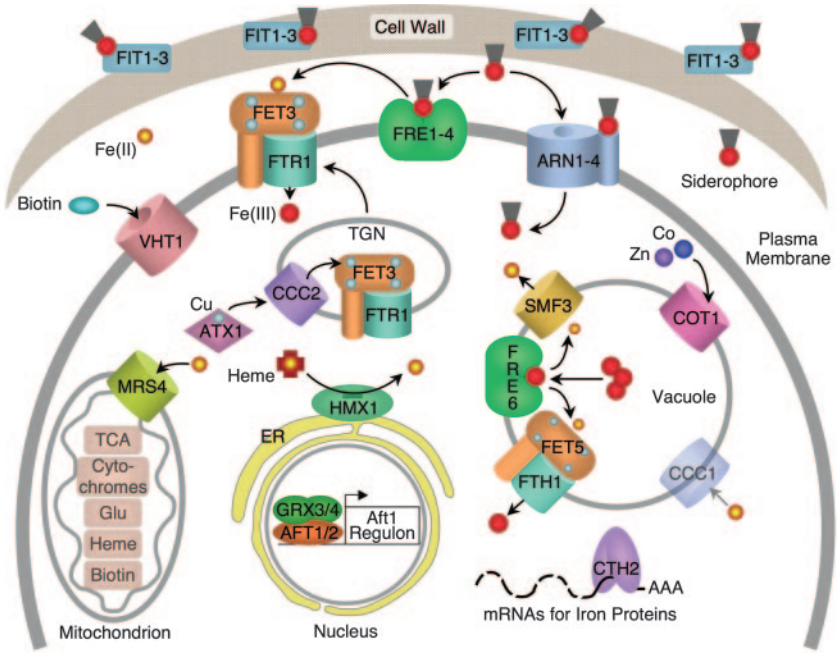

FIG. 2. Response to iron deprivation in *Saccharomyces cerevisiae*. Proteins under the transcriptional control of Aft1p and Aft2p are labeled with black text. Ccc1p, proteins of the tricarboxylic acid cycle, the respiratory cytochromes, and the glutamate, heme, and biotin biosynthetic pathways are down-regulated during iron deficiency and are indicated with gray text.

duced by Aft1p under conditions of iron depletion, while *FRE1* is also induced by copper depletion via Mac1p. Two additional *FRE* family members, *FRE5* and *FRE6*, are also under the control of Aft1p, and Fre6p has been shown to localize to the vacuole, where it functions in the reductive transport of iron and copper from the vacuole to the cytosol (49, 54). A seventh member, *FRE7*, is under the control of the copper-dependent Mac1p transcription factor.

Copper has an important role in reductive iron uptake, and Aft1p induces the transcription of four genes with links to copper. Reduced iron is taken up through a high-affinity transport complex that consists of a multicopper ferroxidase (Fet3p) and a permease (Ftr1p). The oxidase activity of Fet3p is required for iron uptake, and copper is required for the oxidase activity of Fet3p. Copper is inserted posttranslationally into Fet3p in a post-Golgi compartment of the secretory pathway, and the copper chaperone Atx1p and the post-Golgi copper transporter Ccc2p are required for copper insertion. Atx1p and Ccc2p are regulated by iron, not copper, which suggests that they function primarily in iron uptake rather than copper homeostasis. Molecular oxygen is also required for the activity of Fet3p, and under hypoxic conditions, cells express a low-affinity ferrous iron transporter, Fet4p, that serves as an oxygen-independent system of iron uptake (15, 19). A paralogue of Fet3p, termed Fet5p, assembles with a paralogue of Ftr1p, termed Fth1p, to form a ferrous iron transport complex (55, 61) that functions in conjunction with Fre6p at the vacuolar membrane (49, 54). Although *FET5* does not appear to be regulated directly by Aft1p, the remainder of these genes are regulated by iron through Aft1p.

NONREDUCTIVE UPTAKE OF IRON BY SIDEROPHORE TRANSPORT

S. cerevisiae can take up a variety of siderophore-iron chelates through a homologous group of transporters that comprise the ARN/SIT subfamily of the major facilitator superfamily of transporters (reviewed in reference 39). Each of these transporters is predicted to have 14 transmembrane domains and to transport intact iron-siderophore chelates. Each transporter exhibits specificity for a group of fungal and/or bacterial siderophores, and some strain-dependent differences in specificity have been described (Table 2). Some of the siderophore transporters are regulated posttranslationally by their localization within the late secretory pathway. Both Arn1p and Arn3p/Sit1p traffic directly from the *trans*-Golgi network to the vacuole for degradation when their respective siderophore substrates are not present extracellularly (14, 23). Arn1p is recognized at the *trans*-Golgi network by Gga2p, a

TABLE 2. Siderophore substrates of ARN/SIT family of transporters

Transporter	Siderophore substrate	K_t[b] (μM)
Arn1p	Ferrichromes[a]	0.9
Arn2p/Taf1p	Triacetylfusarinine C	1.6
Arn3p/Sit1p	Ferrioxamine B	0.5
	Ferrichromes[a]	2.3
Arn4p/Enb1p	Enterobactin	1.9

[a] Ferrichromes include ferrichrome, ferrichrome A, ferricrocin, ferrichrycin, ferrirhodin, and ferrirubin. Some strain-specific variation in the specificities of Arn1 and Arn3 for different ferrichromes has been reported (18, 28, 67).

[b] K_t, equilibrium transport constant.

clathrin adaptor protein, which directs the transporter to the vacuolar protein sorting pathway (21). Arn1p is then ubiquitinated by Rsp5p and sorted into the multivesicular body for delivery to the vacuolar lumen. Both Arn1p and Arn3p/Sit1p are diverted from the vacuolar sorting pathway to the plasma membrane in the presence of their respective siderophore substrates, and in the case of Arn1p, this relocalization involves the binding of ferrichrome to a receptor domain of the Arn1p transporter itself (14, 22). In contrast, Arn4p/Enb1p traffics directly to the cell surface, even in the absence of its substrate, enterobactin. The relocalization of Arn1p and Arn3p/Sit1p to the plasma membrane only in the presence of their specific siderophore substrates effectively prevents these transporters from moving nonspecific substrates across the plasma membrane when the specific substrate is unavailable. Thus, the cell is protected from the uptake of potentially toxic small molecules. Why does Arn4p/Enb1p not traffic in this pattern? Enterobactin is structurally very dissimilar from the hydroxamate siderophores ferrichrome and ferrioxamine B; perhaps *S. cerevisiae* is exposed to a toxic small molecule that structurally mimics the hydroxamates but not enterobactin and thus the trafficking of Arn1p and Arn3p/Sit1p protects the cell from this specific toxin.

Higher levels of extracellular ferrichrome are associated with both the uptake of ferrichrome and the cycling of Arn1p on and off the plasma membrane (23). Both ubiquitination via Rsp5p and the activity of Gga1p and -2p (C. C. Philpott and Y. Deng, unpublished observations) are involved in the internalization of Arn1p. Although mutations that inhibit actin-dependent endocytosis and the cycling of Arn1p also inhibit the uptake of ferrichrome, mutations in Gga2p and Rsp5, which inhibit cycling of Arn1p and Arn3p without interfering with the endocytosis machinery, do not inhibit uptake of siderophores, indicating that cycling is not required for uptake (14, 21). The internalization of the transporters to a sorting compartment such as the early endosome may allow the cell to selectively recycle the transporters to the plasma membrane for continued uptake or divert them to the late endosomal pathway for vacuolar degradation.

Ferric siderophores are taken up as intact chelates by the ARN transporters, but the iron must dissociate from the siderophore prior to its use by the cell. After uptake through Arn3/Sit1p, intracellular ferrioxamine B accumulates in the vacuole as an intact chelate (14). Whether the iron dissociates from the siderophore within the vacuole or the chelate is transported to the cytosol prior to dissociation is not known. Ferrichrome accumulates as an intact chelate in the cytosol after uptake, indicating that both siderophores can serve as iron storage molecules (34). Iron can be released from cytosolic ferrichrome through the degradation of the siderophore. Although reductive mechanisms for iron release from siderophores have been identified in prokaryotes and evidence for their existence is present in some species of fungi, no reductive mechanism has been identified in *S. cerevisiae*.

THE AFT1P AND AFT2P REGULON: MOBILIZATION OF STORED IRON

Yeast cells can grow in iron-depleted media for several generations, indicating that they express efficient systems for iron storage and mobilization. *S. cerevisiae* and other yeast species do not express ferritin, the major iron storage protein found in prokaryotes and most eukaryotes. Early studies on the distribution of iron in yeast reported that the vacuole is the main iron storage compartment, although some iron is present in the cytosol, mitochondria, and other compartments (47). When iron is abundant in the environment, vacuoles accumulate iron through the activity of the iron and manganese transporter Ccc1p (5, 26). Sequestration of iron within the vacuole protects cells from the toxic effects of iron and allows cells to utilize stored iron when extracellular iron is scarce (29). Strains deleted for *CCC1* cannot maintain proper iron balance and are more sensitive to extremely high and low concentrations of extracellular iron. Although iron-bound ferrioxamine B can accumulate in the vacuole, the molecular form of other iron species deposited in this compartment is less clear. Iron may form complexes with polyphosphates or organic acids present in the vacuole, but this awaits further research.

When extracellular iron levels fall, *CCC1* transcription is shut off, and Aft1p directs the expression of vacuolar proteins that permit the efflux of iron from the vacuole to the cytosol to alleviate iron deficiency. In many ways, these vacuolar proteins duplicate the iron transport systems that are present at the plasma membrane. One member of the FRE family of metalloreductases, Fre6p, is expressed exclusively on the vacuolar membrane, where it is involved in the reduction of vacuolar iron and copper prior to transport into the cytosol (49, 54). Paralogues of the plasma membrane high-affinity ferrous iron transport complex are also present on the vacuolar membrane and are expressed under conditions of iron deficiency. Fet5p and Fth1p are assembled into a complex that is thought to function similarly to the Fet3p-Ftr1p complex (61). Cells lacking Fet5p and Fth1p exhibit activation of the iron deficiency transcriptional program and an impaired transition from fermentation to respiration, a metabolic state that requires more iron. Cells overexpressing Ccc1p accumulate less iron in the vacuole when Fet5p and Fth1p are also overexpressed (54).

Smf3p is another transporter that functions with Fre6p to move iron out of the vacuole (54). Smf3p is a member of the Nramp family of divalent metal transporters, which includes the mammalian iron transporter DMT1. Smf3p is a paralogue of Smf1p and Smf2p. Although Smf1p and -2p function primarily in the uptake of manganese (41), Smf1p is also involved in the transport of ferrous iron at the plasma membrane (6). Smf3p is the only Nramp family member in yeast that is regulated by iron, and it is primarily regulated by Aft2p rather than Aft1p (8, 51). Strains lacking Smf3p exhibit activation of Aft1p target genes and accumulate more iron in the vacuole (54). In contrast, overexpression of Smf3p leads to diminished retention of iron in the vacuole. Smf3p is also induced under conditions of oxygen deficiency and provides the cell with an oxygen-independent mechanism for mobilizing iron stored in the vacuole.

Iron-deficient cells are sensitive to the toxic effects of other transition metals, such as cobalt, copper, zinc, and manganese, and increased uptake of these metals through low-affinity and low-specificity transporters may contribute to this sensitivity. Iron depletion also triggers the Aft1p-dependent expression of Cot1p (53), a transporter on the vacuolar membrane with specificity for metals other than iron. Cot1p is involved primarily in the vacuolar accumulation of zinc and cobalt (7, 31), and its expression during

iron deficiency suggests that sequestration of other metals in the vacuole is an adaptive response (30).

In addition to the vacuolar iron storage pool, cellular iron is also present in substantial quantities in the mitochondria, the site of iron incorporation into heme and ISC. As such, ISC and heme proteins constitute a cellular iron pool that can potentially be mobilized for use during iron deficiency. *HMX1* encodes the yeast heme oxygenase and is actively transcribed during iron deficiency (20, 43). Hmx1p is a heme-degrading enzyme localized to the cytosolic face of the endoplasmic reticulum, and cells lacking Hmx1p exhibit accumulation of intracellular heme, activation of Aft1p, and a reduced capacity to utilize heme as the sole source of nutritional iron. Heme is an important regulatory molecule in yeast and serves as an activator of the Hap1p and Hap2p/3p/4p/5p transcription factors, which control the activation of genes involved in aerobic growth (25). Many of these genes encode components of the respiratory cytochromes, which are very iron-rich. Expression of Hmx1p during iron deficiency leads to degradation of regulatory pools of cellular heme, which leads to reduced Hap1p activation and reduced expression of iron-containing respiratory complexes. Thus, Hmx1p serves a dual purpose in making heme iron available for metabolic needs and decreasing the flux of iron into respiratory complexes.

METABOLIC RESPONSE TO IRON DEPLETION

Examination of the transcriptional response to iron depletion suggests that yeast cells respond to iron depletion by altering their utilization of iron, shifting it away from nonessential metabolic pathways while preserving essential ones. Several lines of evidence support this hypothesis. *S. cerevisiae* can metabolize the products of glycolysis through either fermentation or respiration; however, strains grown in iron-depleted media or strains lacking Aft1p or Fet3p are respiration deficient and cannot grow on nonfermentable carbon sources. DNA microarray analysis of yeast cells grown under conditions of iron starvation versus iron excess reveals that mRNA levels for many proteins in metabolic pathways that contain heme-proteins and ISC proteins are decreased in iron-depleted cells (44, 53). These pathways include the tricarboxylic acid cycle, the mitochondrial respiration and electron transport chain, and the heme and biotin biosynthetic pathways. Transcripts coding for iron-sulfur proteins involved in the synthesis of leucine, glutamate, and lipoic acid are also diminished. Multiple mechanisms are involved in the down-regulation of these pathways.

Cth2p/Tis11p is an Aft1p target that is strongly induced by iron deficiency and mediates many of the changes in transcript levels observed during iron deficiency (44). Cth2p and its paralogue Cth1p are members of the tristetraprolin family of RNA binding proteins and recognize AU-rich elements in the 3′ untranslated region (3′-UTR) of specific mRNA transcripts. Binding of Cth2p to these AU-rich elements leads to destabilization and degradation of the transcripts. Many of the transcripts that are decreased under iron deficiency contain these AU-rich elements and show elevated levels in a strain lacking Cth2p. Cth2p has been shown to specifically bind to the AU-rich element present in the 3′-UTRs of *SDH4* and *ACO1* mRNAs, which encode the heme-binding subunit of succinate dehydrogenase and the ISC enzyme aconitase, respectively. Thus, the set of genes activated by Aft1p during iron deficiency includes a protein that specifically down-regulates transcripts involved in the utilization of iron. This allows the cells to divert iron that would ordinarily be incorporated into these pathways to other, essential pathways. For example, nine genes of the ergosterol and fatty acid biosynthetic pathways are up-regulated during iron deficiency. Since both of these essential pathways contain iron-dependent enzymes, increased expression during iron deficiency may represent a homeostatic mechanism triggered by a decrease in the flux through these pathways.

Another example of the shift to iron-independent metabolism in the setting of iron deficiency is illustrated by the regulation of biotin acquisition. Although technically auxotrophic for biotin, *S. cerevisiae* can synthesize biotin from 7-keto, 8-amino-, and 7,8-diamino-pelargonic acid precursors and from desthiobiotin (38). The ultimate step in biotin biosynthesis is catalyzed by Bio2p, an ISC protein (60). The biotin biosynthetic enzymes Bio3p, Bio4p, and Bio2p are expressed in cells grown in iron-replete medium but are shut off at the transcriptional level under conditions of iron deficiency (53). Iron deficiency also triggers the expression of the Aft1p target gene *VHT1*, which encodes the transporter required for high-affinity biotin uptake at the cell surface (57). Cells lacking Vht1p can survive by synthesizing biotin de novo when cells have adequate levels of iron, but they cannot synthesize sufficient biotin to support growth under conditions of iron deficiency. Thus, yeast cells reciprocally regulate biotin uptake and biosynthesis, relying exclusively on uptake when iron is limited and shifting to synthesis only when iron is plentiful. The transcription of the specific transporter for the pelargonic acid precursors, Bio5p, is also increased under conditions of iron deficiency (1), which may indicate that cells can store biotin precursors for later biosynthesis.

Nitrogen assimilation pathways also appear to be altered in response to changing iron availability. Several genes involved in amino acid and nitrogen uptake and metabolism are up-regulated under conditions of iron deprivation, and this up-regulation may be due to changes in glutamate synthesis during iron deficiency (53). All of the nitrogen-containing compounds in yeast are synthesized from the amino acids glutamate and, to a lesser extent, glutamine, and yeast cells have two pathways for synthesizing glutamate from ammonium, the preferred nitrogen source (32). Glutamate is synthesized directly from ammonium and 2-oxoglutarate in a reaction that requires NADPH. Two isoforms of glutamate dehydrogenase, Gdh1p and Gdh3p, catalyze this reaction. A second pathway involves a two-step process in which glutamine synthetase (Gln1p) catalyzes the formation of glutamine from ammonium and glutamate and then glutamate synthase (Glt1p) catalyzes the formation of two glutamate molecules from glutamine and 2-oxoglutarate. The summation of the second, two-step pathway is identical to the first, except that ATP and NADH are consumed rather than NADPH. Glt1p is an ISC enzyme (33), and both glutamate synthase activity and *GLT1* mRNA levels decrease in the setting of iron deprivation. Thus, cells shift to an iron-independent pathway for glutamate synthesis under conditions of iron deficiency.

The genes required for purine biosynthesis are also down-regulated during iron deficiency, and although purine biosynthesis is an iron-dependent process in most organisms, there are no

known iron-dependent enzymes in this pathway in *S. cerevisiae* (53). Expression of the purine biosynthetic pathway is regulated homeostatically through the production of ADP and ATP, allosteric inhibitors of Ade4p, and by the transcription factors Bas1p and Pho2p, which are activated by intermediates of the biosynthetic pathway (11, 48). Iron appears to exert its effects upstream of Bas1p and Pho2p, but mechanistically, the effects of iron deprivation on purine biosynthesis remain unclear.

REVERSAL OF THE IRON DEFICIENCY RESPONSE

Expression of the genes involved in the response to iron deficiency serves to maintain cellular iron homeostasis and to optimize the metabolism of iron-deficient cells. Less is known regarding the response to acute increases in extracellular iron, but *S. cerevisiae* has the capacity to quickly reverse the iron deficiency response when cells become iron replete. Aft1p is quickly inactivated in iron-replete cells, and levels of *FET3* mRNA are undetectable within 30 min of adding iron (C. C. Philpott, unpublished observations). Rnt1p contributes to the rapid degradation of mRNA transcripts encoded by Aft1p-regulated genes. Rnt1p is a double-stranded RNA endonuclease of the RNase III family. Cells lacking Rnt1p are sensitive to iron and exhibit inappropriately elevated levels of Aft1p target mRNAs under iron-replete conditions (27). Aft1p target proteins are also regulated posttranslationally. High levels of ferrous iron uptake at the cell surface lead to internalization and degradation of the Fet3p/Ftr1p transporter complex, which tend to limit the amount of iron taken up when cells encounter an iron-rich environment (12). Arn1p, the ferrichrome transporter, does not exhibit a similar siderophore-mediated degradation. This stability in the setting of ferrichrome accumulation may be due to the capacity of ferrichrome to serve as an intracellular iron storage molecule.

CONCLUSION

Studies with yeast indicate that in the face of limitation in iron availability, cells not only will increase their uptake of iron and their mobilization of stored iron but also will adjust their metabolism to more efficiently use the iron that is available. The remarkable flexibility in the use of this essential nutrient reveals a metabolic hierarchy within the cell, with iron shifting away from nonessential pathways and into more essential ones.

The picture of how cells respond to changes in iron is not completely clear, however. The molecular form of iron that is sensed by the cell and how that sensing results in altered Aft1p/2p activity remain unclear. Although several proteins involved in general transcriptional repression and activation influence the expression of Aft1p target genes, we do not yet understand the role of chromatin remodeling in the transcriptional response to iron deficiency and how chromatin remodeling and the activity of specific transcription factors are coordinated. Iron triggers the Aft1p-independent activation of some genes, and the specific transcription factors involved in this activation have yet to be identified.

Many of the studies described here have focused on changes in the levels of transcripts and proteins involved in iron homeostasis and the response to iron deficiency. Yet alterations in protein levels do not always result in the predicted changes in transport activity or flux through a metabolic pathway. Despite increased expression of reductases and the high-affinity iron transport complex during growth in iron-poor media, cells accumulate less iron in this setting. Similarly, subtle changes in the expression of iron-containing enzymes may lead to significantly more or less iron being devoted to a particular enzymatic pathway, but the flux of metabolites through this enzymatic pathway may change little, if at all, as flux is determined primarily by enzyme kinetics and the concentrations of enzyme substrates. Although the flux of metabolic intermediates through the respiratory chain in iron-deficient cells is not sufficient to support the growth of cells, it is not clear that this phenomenon is fully explained by changes in levels of the respiratory complexes. The extent to which flux through metabolic pathways and the products of biosynthetic pathways are maintained or altered in the face of falling iron availability will be revealed by studies of metabolite levels and the extent to which homeostasis is achieved in iron-deficient cells. Examination of the yeast response to iron deficiency will also yield clues as to how other organisms might prioritize their use of iron at the cellular level.

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